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Modulating MicroRNAs in Cardiac Surgery Patients: Novel Therapeutic Opportunities?

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Abstract

This review focuses on microRNAs (miRs) in cardiac surgery, where they are emerging as potential targets for therapeutic intervention as well as novel clinical biomarkers. Identification of the up/down-regulation of specific miRs in defined groups of cardiac surgery patients can lead to the development of novel strategies for targeted treatment in order to maximise therapeutic results and minimise acute, delayed or chronic complications. MiRs could also be involved in determining the outcome independently of complications, for example in relation to myocardial perfusion and fibrosis. Because of their relevance in disease, their known sequence and pharmacological properties, miRs are attractive candidates for therapeutic manipulation. Pharmacological inhibition of individual miRs can be achieved by modified antisense oligonucleotides, referred to as antimiRs, while miR replacement can be achieved by miR mimics to increase the level of a specific miR. MiR mimics can restore the function of a lost or down-regulated miR, whilst antimiRs can inhibit the levels of disease-driving or aberrantly expressed miRs, thus de-repressing the expression of mRNAs targeted by the miR. The main delivery methods for miR therapeutics involve lipid-based vehicles, viral systems, cationic polymers, and intravenous or local injection of an antagomiR. Local delivery is particularly desirable for miR therapeutics and options include the development of devices specific for local delivery, light-induced antimiR, and vesicle-encapsulated miRs serving as therapeutic delivery agents able to improve intracellular uptake.

Here, we discuss the potential therapeutic use of miRNAs in the context of cardiac surgery.

Keywords: MicroRNA, cardiac surgery, therapeutic, cardiac protection, angiogenesis,

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Abbreviations

AAA = abdominal aortic aneurysm

AKI = acute kidney injury

AMI = acute myocardial infarction

BAV = bicuspid aortic valve

CABG = coronary artery bypass graft

CAD = coronary artery disease

CHD = congenital heart disease

CPB = cardiopulmonary bypass

HF = heart failure

HLHS = hypoplastic left heart syndrome

IHD = ischemic heart disease

LNA = locked nucleic acid

lncRNA = long non-coding RNA

LV = left ventricle

LVAD = left ventricular assist device

LVEF = left ventricular ejection fraction

MI = myocardial infarction

miR = microRNA

MMP = metalloproteinase

PAH = pulmonary arterial hypertension

RNA = ribonucleic acid

RV = right ventricle

RVOT = right ventricular outflow tract

SAVR = surgical aortic valve replacement

TAVR = transcatheter aortic valve replacement

TGA = transposition of the great arteries

ToF = tetralogy of Fallot

1. Introduction: blooming microRNA research

Since the human genome project mapped the first chromosome in 1999, it was observed that only about 20,000 genes are protein coding (Pheasant and Mattick, 2007). In fact, the majority of DNA is transcribed into non-coding ribonucleic acids (ncRNAs). ncRNAs are functional RNA molecules that are not translated into proteins and work by regulating gene expression at the transcriptional and/or post-transcriptional level. The discovery of ncRNAs dates back 50 years, with the characterisation and sequencing of alanine transfer-RNA (Holley et al., 1965). More recently, the ENCODE project ("The International Encyclopedia of DNA Elements") shed further light on the biochemical activity of the human genomic DNA (ENCODE Project Consortium, 2012), revealing that only a fraction of the human genome carries the codes for protein synthesis (Ponting and Hardison, 2011).

Depending on the number of nucleotides, ncRNAs have been classified as either 'short' (< 30 nucleotides) or 'long' (> 200 nucleotides). Amongst the short non-coding RNAs, further distinctions can be made between different classes, including microRNAs (miRNAs, or miRs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs). The other class of long ncRNAs (lncRNAs) is currently being investigated for its function and involvement in biological phenomena such as transcriptional enhancement, gene imprinting, and dosage compensation of sex chromosomes (Mercer et al., 2009; Quinn and Chang, 2016).

MicroRNAs were first discovered in the 1990s (Lee et al., 1993; Wightman et al., 1993). The miR biogenesis begins with a long 5'-capped and Poly A tailed primitive miRNA (pri-miR) transcript derived from protein coding genes or an independent non-coding transcriptional unit being configured into a hairpin structure. These miR-producing transcripts can contain single miRs or form polycistronic miR clusters. The process of maturation of pri-miRs begins in the cell nucleus, leading to the production of a precursor miR (pre-miR), which in turn is transported into the cytosol or the endoplasmic reticulum to be processed into its approximately 22-nucleotide long mature form by Dicer, a RNase III endonuclease. The resulting structure is typically referred to as "hairpin" and presents itself as double stranded,

of which one is incorporated into the RNA-induced silencing complex (RISC) where the miR and the mRNA target interact (Cai et al., 2004; Ha and Kim, 2014; Rodriguez et al., 2004).

The majority of miRs are located intracellularly, but a large number has also been identified in the extracellular space (Cortez et al., 2011; Etheridge et al., 2011). Extracellular miRs found circulating in the bloodstream were observed to be remarkably stable (Creemers et al., 2012; Mitchell et al., 2008). In addition to bloodstream, cell-free miRs have found in other body fluids, including the urine (Weber et al., 2010).

It has been suggested that miRs contribute to almost all developmental and pathological processes in animals, including embryonic development and post-natal regeneration (Colas et al., 2012; Ha and Kim, 2014; Porrello, 2013; Stepicheva and Song, 2016; Wang et al., 2016; Yan and Jiao, 2016). Recently, miRs have been established as key players in the regulation of several cellular processes and in the pathogenesis of different diseases. Biomedical research in the field of miR has flourished, leading to important discoveries implicating roles for miRs in several clinical scenarios, including cancer (Naidu and Garofalo, 2015; Ragusa et al., 2015; Thomas et al., 2015), liver (Lambrecht et al., 2015; Zarfeshani et al., 2015), and skin conditions (Lai and Siu, 2014; Mancini et al., 2014). Of particular interest here, miRs contribute to the embryonic development of the heart, normal cardiovascular function and cardiac pathophysiology (Cai et al., 2010; Catalucci et al., 2008; Liu and Olson, 2010; Thum et al., 2008; van Rooij and Olson, 2007a), including stress response mechanisms and tissue remodelling (van Rooij and Olson, 2007b; van Rooij, 2011). Since their discovery and the earlier stages of cardiovascular research, deregulation of cardiac- and vascular- expressed miRs has been functionally associated with the development of heart and vascular diseases (Romaine et al., 2015). Consequently, therapeutic targeting of miRs has been proposed as a novel approach to prevent and cure cardiovascular diseases and cardiovascular complications of metabolic disease (van Rooij and Olson, 2007b).

This review focuses on translational efforts on miRs in the context of cardiac surgery, where miRs represent potential biomarkers and therapeutic targets. Worldwide, there were over 17.5 million deaths from cardiovascular disease in 2012 (www.who.int). Cardiac surgery broadly refers to surgical procedures on the heart or the great vessels that are performed to treat ischemic, valvular, rheumatic heart disease of congenital or acquired nature, including heart transplant. Thousands of heart surgeries are performed every day worldwide, and more than 2,300 people receive a heart transplant each year in the USA alone (<http://www.cdc.gov>, <http://bluebook.scts.org>, <http://www.texasheart.org>), with enormous economic impact (Weiser et al., 2008). Furthermore, the number of surgical procedures was estimated to increase by as much as 50% between 2006 and 2025, assuming stable incidence rates, and even with significant decreases in treatment rates, the number of procedures per year was still estimated to increase due to global population growth and aging (Etzioni and Starnes, 2011).

In the context of cardiovascular pathology, identification of the mechanistic role of a single or multiple groups of miRs will have a descriptive value and enhance our understanding of pathophysiology. Perhaps even more exciting would be the identification of the deregulation of specific miRs in specific classes of cardiac surgery patients. This could, potentially, lead to the development of innovative therapeutic strategies for targeted treatment able to maximise the therapeutic results by slowing or preventing recognised pathological processes, and minimising acute or chronic complications. MiRs could also be involved in determining the outcome independently of complications, for example in relation to angiogenesis (perfusion) and fibrosis (heart, vasculature and graft remodelling). Moreover, miRs could translate to new prognostic and predictive biomarkers in cardiac surgery, with potentially important applications, such as to help predict optimal timing for surgical intervention before ventricular dysfunction occurs, or to risk stratify patients for the development of complications after surgery. This review will discuss the potential impact of miRs research in acquired and congenital cardiovascular surgical pathologies.

2. Cardiovascular surgery

2.1. The ischemic heart and coronary artery bypass surgery

Coronary heart disease or ischemic heart disease (IHD) is the leading cause of death worldwide, with an estimated 1/6 men and 1/10 women dying from IHD per year, and 2 million people in the UK alone presenting with angina, its most common symptom (<http://www.nhs.uk>). Essentially, ischemia is caused by a reduction of blood flow to the myocardium due to a pathological narrowing caused by atherosclerotic plaque in the coronary arteries, which can lead to myocardial infarction (MI) and heart failure (HF). Surgical treatment of ischemic heart disease typically involves coronary artery bypass graft (CABG) (Diodato and Chedrawy, 2014), while support of the failing heart can necessitate the use of different ventricular assist devices (VADs) or even heart transplant.

MiRs could contribute to biological processes underlying heart ischemia and to post-surgery heart adaptation, thus representing novel therapeutic targets. Different miRs have indeed been shown to be involved in the process of atherosclerotic plaque formation, from endothelial cell activation (miR-21) to plaque angiogenesis (miR-92a, miR-27) to fibrous cap stabilisation (miR-143/145) (Caroli et al., 2013). MiR-15a and -15b have been particularly indicated to be involved in mechanisms of ischemia and HF, and therefore represent possible therapeutic target. Inhibition of miR-15-a/b reportedly prevents ischemia-induced cardiomyocyte apoptosis (Small et al., 2010) and increases cardiomyocyte cell cycle with the potential for cardiac repair (Porrello et al., 2011). Another miR that has been suggested to play a crucial role in the possible treatment of IHD is miR-210. This is known as the “hypoxia-miR” because it is induced by hypoxia in different cell types (Chan and Loscalzo, 2010; Fasanaro et al., 2008). MiR-210 is also expressed in endothelial cells, where it induces angiogenesis, and cardiac myocytes, where it contrasts apoptosis (Mutharasan et al., 2011). Vesicle-mediated miR-210 delivery to the ischemic mouse heart resulted in a beneficial effect on global cardiac function, with pressure-volume loop measurements revealing overall more favourable left ventricular remodelling (Hu et al., 2010).

Other animal studies reaffirm the role miRs are likely to play in IHD, which in animals is usually modelled *via* left descending coronary ligation to induce MI. Based on murine MI models, important effects were noted following treatment with inhibitors for miR-21 (Gu et al., 2015), miR-150 (Liu et al., 2015), miR-99a (Li et al., 2014), miR-24 (Meloni et al., 2013), and miR-130a (Lu et al., 2015). Beneficial changes post-MI included notable improvements in left ventricular (LV) ejection fraction and fractional shortening, reduction of the infarcted area, improved angiogenesis, decreased cellular apoptosis, and a reduction of collagen deposition in the infarcted area, thereby suggesting therapeutic potential and a possible cardio-protective role post-MI in terms of LV remodelling

Postoperative MI occurs in 2-15% of patients following cardiac surgery (Al-Attar, 2011). Some miRs reportedly stimulate cardiomyocyte proliferation, with the possibility to promote post-MI cardiac regeneration (Eulalio et al., 2012). *In vivo* work has shown improvements in cardiac function and remodelling after adeno-associated virus (AAV)-mediated administration of miR-199a and miR-590 (Eulalio et al., 2012). Also from a therapeutic perspective, the possibility of improving/restoring the contractility of the myocardium is appealing especially in patients following CABG, when myocardial stunning typically occurs (Leung, 1993). Targeting miRs involved in controlling contractility and improving intracellular calcium handling could represent a possible therapeutic target (Wahlquist et al., 2014). In this case, miRs involved in suppressing intracellular calcium handling have been recognised by means of high-throughput functional screening of the human miRNAome. In particular, it was observed that miR-25 contributes to worsening heart performance post HF, thereby representing a possible therapeutic target. This was confirmed by testing the effect of an antisense oligonucleotide against miR-25 in a model of HF and consequently observing an improvement in heart function in mice (Wahlquist et al., 2014). However, the role of miR-25 and the associated therapeutic potential is not conclusive at present. For instance, *in vivo* inhibition of miR-25 has also been reported to lead to spontaneous cardiac dysfunction, sensitizing the myocardium to HF (Dirkx et al., 2013). While further research is needed to elucidate the role of miR-25, it has been suggested that this specific miR could have a

beneficial role in acute HF, however it may lead to maladaptive effects in the long term (Bush and van Rooij, 2014).

The failing heart may also require support, as a therapy or bridge to transplant (Holley et al., 2014) in the form of a LVAD. LVAD support has been shown to be associated with a decrease in the expression of miR-1, miR-133a and miR-133b in the myocardium of patients with dilated cardiomyopathy and an increase in expression in patients with IHD, irrespective of the duration of mechanical support, despite a wide range of 57-557 days (Schipper et al., 2008).

The role of miRs has also started to be explored in the context of heart transplantation, with a study looking at a group of heart transplant recipients (n=113) and observing a differential miR expression in patients rejecting allografts (Duong Van Huyen et al., 2014). In particular, four circulating miRs (miR-10a, miR-31, miR-92a, and miR-155) were found to be good discriminators of patients with and without allograft rejection. The importance of this observation lies in the possibility of monitoring patients post-transplant and distinguishing sub-groups of patients that could be at a higher risk for toxicity and/or adverse events, thereby improving organ donor-recipient matching. Moreover, future studies could say whether these miRs are also functionally involved in transplant rejection, thus representing therapeutic targets for devising miR drugs that would reduce/prevent allograft rejection (Batkai and Thum, 2014).

Another high impact therapeutic application for IHD patients that require CABG surgery is represented by the possibility of targeting specific miRs to control vascular cells and stem cells employed for tissue-engineering of seeded vascular conduits instead, or in addition to, vein grafts, in order to improve graft patency (Caputo et al., 2015a). Tissue engineering small vascular grafts is a challenging but clinically important field of research.

In summary, it is accepted that miRs play a role in the ischemic, infarcted and failing heart and its surgical management, a role that could, potentially, be pivotal (Leite-Moreira et al., 2013) for both diagnostic and therapeutic innovations.

2.2. Surgical aortic valve replacement

Aortic stenosis is the prevalent valvular disease in Western countries (Osnabrugge, 2013), with an estimated 67,500 surgical aortic valve replacements (AVR) being carried out every year in the USA alone (Clark et al., 2012). Following AVR, it has been shown that aortic stenosis patients with impaired LV function exhibit a marked functional improvement (Sharma et al., 2004). In the past ten years, a percutaneous alternative to AVR (transcatheter aortic valve replacement, TAVR) has been shown to yield excellent results or equivalent results to the surgical option (Hamm et al., 2015; Nagaraja et al., 2014). However, TAVR is still typically offered to patients with high surgical risk. Additional knowledge and possible innovative therapeutics for AVR are therefore still highly desirable and the role of miRs has been explored also in this context.

One study discussed the role of miR-133a in the process of reverse LV remodelling following pressure release in aortic stenosis patients (n=74), based on measurements from LV biopsies and plasma (García et al., 2013). MiR-133 has been associated with cardiac hypertrophy (Carè et al., 2007), playing a role in regulating apoptosis, fibrosis, and potassium channel remodelling (Abdellatif, 2010). Another study (Yanagawa et al., 2012) investigated the role of miR-141 as a regulator of aortic valve calcification, analysing tissue from aortic valve leaflets collected at the time of surgery and comparing two groups of patients, i.e. bicuspid aortic valve (BAV) vs. tricuspid aortic valve. A substantial and statistically significant reduction (nearly 15 fold) in miR-141 was observed in the BAV leaflets, suggesting that this miR could have therapeutic potential especially in BAV patients. This is likely related to the *in vitro* observation that miR-141 represses valvular interstitial cell response to osteogenic stimuli blocking BMP2-dependent calcification (Yanagawa et al., 2012). A third study (Zhang et al., 2014) used a similar approach, comparing valve tissue samples from AVR patients and heart donors with non-calcified valves (n=10 per group), and concluded that miR-30b could be relevant in preventing – even – arresting aortic valve calcification. The latter can indeed lead to AVR and valve failure after transplantation, and

thus the development of miR-based therapeutics for preventing bio-prosthetic calcification could be a clinically important application.

2.3. Surgical repair of aneurysmal aorta

Aneurysms (i.e. dilatations) can form in the ascending and descending thoracic aorta and in the abdominal aorta, with 80% of aortic aneurysms typically form in the infra renal abdominal aorta (Aggarwal et al. 2011). The major risk of aortic aneurysms is related to their potential complication of rupture or dissection, which is associated with a high mortality. Even uncomplicated aneurysms would require clinical intervention or surveillance. Endovascular stent graft treatments have revolutionised the treatment of abdominal aortic aneurysms (AAA) (Schanzer and Messina, 2012), although associated with higher re-intervention rate and more complications than open surgical repair (Wilt et al., 2006). The treatment carries lower risk upfront with no difference in long-term mortality and cost (IMPROVE Trial Investigators et al., 2014).

Regardless of treatment approach and location of the aortic aneurysm, miRs are likely to be involved in different regulatory mechanisms that can lead to the dilatation of the thoracic and abdominal aortic wall, in relation to proliferation, migration and phenotypic transformation of vascular smooth muscle cells (Duggirala et al., 2015).

Aneurysm in the ascending thoracic aorta differ (etiologically) from AAA, with a considerable proportion being linked to genetic diseases or hereditary causes and altered flow from the aortic valve. Genetic alternations have been discussed in syndromes such as Marfan, Loeys-Dietz, and Ehler-Danlos, or in association with BAV (Bisleri et al., 2013). Changes in miR expression have been reported in thoracic aortic aneurysms (Ikonomidis et al., 2013). This study compared ascending aortic tissue and plasma samples patients with BAV (n = 21) and tricuspid aortic valve (n = 21) at the time of surgery, all patients presenting with thoracic aortic aneurysm, as well as from controls without aortopathy (n=10). Levels of miR-1 and miR-21 varied significantly between bicuspid and tricuspid valve samples, and differences were observed between aneurysmal and normal aortas. These observations are

supported by another study (Jones et al., 2011) that found a significant inverse association between miR expression levels for miR-1, -21, -29a, and -133a, and aortic diameter (i.e. increasing aortic diameter related to decreased miR expression). A link was also established between miR expression and metalloproteinases MMP-2 and MMP-9, which are proteins involved in aneurysm development (Jones et al., 2011). Furthermore, expressional changes in miRs belonging to either the miR-29 or miR-30 families were observed in thoracic aortic dissection when comparing non-aneurysmal and non-dissected thoracic aorta, suggesting a miR contribution not only in aneurysm development but also the threatening consequence of aortic dissection (Liao et al., 2011).

Observations gathered from studies performed in AAA and descending thoracic aorta, which are more likely to receive endovascular treatment, could be illuminating also for the case of dilated ascending/thoracic aortas requiring surgical intervention. One miR likely to be involved in aortic aneurysm formation is miR-29. It has been reported that miR-29 affects the expression of extracellular matrix (collagens, elastin, fibrillins) and miR-29 inhibition by means of anti-miRs mitigates aneurysm formation in an experimental mouse models (Boon and Dimmeler, 2011; Zampetaki et al., 2014). The latter observation leads to envisage possible clinical benefit of inhibition of miR-29 inhibition in patients with aortic aneurysm (Boon and Dimmeler, 2011).

Although AAA is more frequent, more patients die from complications related to undetected thoracic aortas. BAV patients are more at risk of developing ascending aortic aneurysms at a younger age. These aneurysms seem to rupture at a lower diameter. This biological behaviour pattern is inconstant in BAV suggesting an interplay between genetics regulation and blood flow. It is in this class of patients in particular that miRs might represent new biomarkers, by providing us with tools to predict behaviour and, most exciting of all, potentially represent minimally invasive therapeutic solutions to slow or arrest the development of aneurysms.

2.4. Surgical repair of congenital heart disease

Congenital heart disease (CHD) surgery represents a particularly relevant area with substantial potential for future research in novel biomarkers and therapeutic solutions. CHD accounts for the most common birth malformations, with estimates up to one third of all major congenital anomalies (Hoffman and Kaplan, 2002; van der Linde et al., 2011). The causes for CHD can be genetic, environmental or a combination of the two, and the role of miRs in CHD development represents a fertile ground for new studies. Previous research has shown miR-1 to be fundamental for embryonic heart development and that dysregulation of miR-1 and other important miRs can have developmental consequences, resulting in CHD (Bruneau, 2008). With the advent of high-throughput genomic technologies and a systems biology approach it will be possible to gather further knowledge of isolated, non-syndromic CHD, and this could have potential ramifications in three crucial areas –diagnosis, monitoring and therapy (Vecoli et al., 2014).

An interesting study (Ma et al., 2015) investigated the potential role of miRs in pulmonary arterial hypertension (PAH) secondary to CHD, albeit on a small group (n=12) of CHD patients. Amongst the several miRs that differed significantly between the patients who developed PAH and those who did not, the expression level of miR-27b correlated with preoperative mean pulmonary arterial pressure. In vitro, overexpression of miR-27b decreased the protein expression of NOTCH1. Despite its limitation of a small sample size, this study indicates that miRs could also be involved in the regulation of PAHsecondary to CHD.

With regards to surgical practice for repairing congenital defects, albeit this remains a largely unexplored and exciting area of study, there has been preliminary research looking into particularly relevant and challenging surgical scenarios. Firstly, BAV is the most common congenital heart disease, accounting for 1.3% births (Hoffman and Kaplan, 2002; Michelena et al., 2015). While linked with ascending aortic dilatation (see Section 7), it is worth mentioning BAV in the context of CHD, as indeed the principal cause of aortic stenosis and insufficiency. A small human study (Nigam et al., 2010), examining fused and unfused aortic valve leaflets from surgical patients, set out to investigate possible underlying differences

between the aortic valve stenosis and aortic valve insufficiency patients. Whilst the study was underpowered, it is interesting to observe it highlighting again the role of miRs in this context, with miR-26a, miR-30b, and miR-195 being decreased in the valves of the aortic stenosis patients in comparison to those requiring surgery due to insufficiency. The mechanism underlying these differences might be partly related to the effect of specific miRs on calcification, which should be explored in larger studies.

Secondly, tetralogy of Fallot (ToF) is the most common cyanotic congenital heart defect (Downing and Kim, 2015). While aspects of the physiology are now well appreciated by the congenital cardiac surgery community (Carminati et al., 2015), underlying mechanisms specific to the development of the disease require further elucidation. MiR research remains rather descriptive and only very few studies have tackled the genomic analysis of such CHD. One study (O'Brien et al., 2012) examined miR expression in RV myocardium from ToF infant patients (n=16, < 1 year of age), foetal samples (n=3, approximately 90 day gestation) and tissue from normally developing infants (n=8, aged-matched, expired due to non-cardiac-related causes). This study highlighted a striking shift in the miR expression, suggesting fundamental differences in cell biology underlying the development of ToF. Interestingly, some of the miRs associated with heart development such as miR-1 and miR-133 were not expressed differently in the ToF samples. A second study (Zhang et al., 2013a) also aimed to investigate possible abnormal miR expression in ToF, and analysed myectomy right ventricular outflow tract (RVOT) tissues. Again, the study population is extremely small (n=5 ToF patients vs. n=3 age-matched controls). Several miRs were expressionally dysregulated in RVOT tissues from the ToF group, and this observation was corroborated by in vitro experiments which specifically indicated miR-424/424* and miR-222, possibly involved in cardiomyocyte proliferation and migration.

Thirdly, transposition of the great arteries (TGA) represents an important conotruncal anomaly involving challenging surgical repairs, whether the historical Senning/Mustard procedures (Love et al., 2008) or more recently the arterial switch operation (Villafañe et al., 2014). One study targeted TGA patients (Tutarel et al., 2013) and specifically sought to

investigate the possible role of miR-423-5p, as an HF biomarker, with the rationale of investigating a group of patients presenting with a systemic RV and reduced EF. While this study analysed a slightly larger number of samples (n=41 TGA patients, n=10 age/sex-matched controls), it was inconclusive, as levels of circulating miR-423-5p did not vary between patients and controls.

Finally, hypoplastic left heart syndrome (HLHS) is a rare defect, requiring complex staged surgical palliation (Feinstein et al., 2012). Newborns with HLHS present with a rudimentary or absent LV and rely on a single systemic RV. One recent study investigated the miR profile in HLHS (Sucharov et al., 2015). The analysis was based on RV tissue samples from HLHS patients (n=15) and controls (n=6), and it should be noted the HLHS patients were at different stages of palliation. A distinct miR profile was observed in HLHS, also including miRs that have been shown to be regulated in HF, such as miR-29b, miR-130a and miR-499. Interestingly miR expression was found to vary with stage of surgery (Norwood vs. Fontan completion), and a possible explanation for such a difference could lie in volume unloading and a response to such a physiological change. However, conclusions should be drawn with caution given the small sample size.

Given the known association between neurodevelopmental complications and CHD (Peyvandi et al., 2016) and the identified role of miRs in neurodevelopmental disorders (Sun and Shi, 2015), it is possible that miR-based therapeutic solutions could also be devised to reduce neurodevelopmental complications in CHD patients undergoing cardiac surgery.

Whilst CHD studies can suffer from small sample sizes, this area is likely to hold great promise for future research, including possible targeting therapeutics, whether at the level of myocardial function or tissue properties and changes in vessels distensibility, known to occur in several CHDs, or post-operative neurological complications. Overall, miRs could certainly represent “a new piece in the paediatric cardiovascular disease puzzle” (Omran et al., 2013).

3. MicroRNAs involved in mechanisms relevant for cardiac surgery patients

3.1. Myocardial protection

The identification of ways to protect the heart from ischaemia- and reperfusion-induced injury is essential for surgery, and has been identified as one of the greatest challenges of cardiology (Piper and Garcia-Dorado, 2009). A mechanism for protecting the myocardium that has been extensively explored consists of alternating short (induced) episodes of sublethal ischemia and reperfusion, either of the heart or at distance (in a leg/arm). This manoeuvre results in the “ischemic preconditioning” (IPC) of the heart in preparation for prolonged ischemia later (Hausenloy et al., 2015; Murry et al., 1986).

It has been suggested that miRs targeting could be a promising therapeutic strategy to restore damaged myocardium and promote cardiac protection, and several specific miRs have been studied in this context. For example, miR-1 has been suggested as an appealing target in regulating cell apoptosis (Yang et al., 2007). MiR-29 has also been indicated as a player in the ischaemia- and reperfusion-induced injury scenario, as a repressor of collagen expression and negative regulation of anti-apoptotic genes, overall suggesting a protective mechanism (Fan and Yang, 2015; Thum et al., 2007; van Rooij et al., 2008).

An interesting study was designed to investigate the hypothesis that miRs could pass the “preconditioned phenotype” from heart to heart. MiRs were extracted from hearts of mice that had received myocardial ischaemia- and reperfusion (I/R) and were incubated with polyamine to form miRNA-amine or miRNA inhibitor-amine complexes, thereby facilitating the entry of the miRNAs into cells after cardiac injection. The miRs were injected in vivo into the left ventricular wall of mice 48 hours before subjecting their hearts to I/R. MiR-1, miR-21 and miR-24 increased in those mice that received “preconditioned miRs”. An observed reduction in infarct size compared to untreated controls suggested that the injection of some or all the preconditioning-derived miRs protected the hearts against further injury (Yin et al., 2009).

One possible mechanism to explain the beneficial role of miRs in this context refers to their role in the regulation of calcium signalling in ischemic/reperfusion injury (Choi et al., 2014), as regulation of intracellular calcium is considered to be therapeutically relevant (Garcia-Dorado et al., 2012). MiR-25 leads to an impairment in calcium uptake and aggravates HF

by interacting with SERCA2a mRNA, whereby inhibition of miR-25 could have therapeutic potential in HF (Wahlquist et al., 2014). Furthermore, it has been indicated that miR-25 can decrease mitochondrial calcium uptake through selective mitochondrial calcium uniporter downregulation (Marchi and Pinton, 2013). Another study discussed the role of miR-145 in the inhibition of calcium overload and calcium-related signalling, and how the overexpression of miR-145 can be protective against cardiomyocyte apoptosis (Cha et al., 2013). Another miR that might be implicated in this context is miR-214, which has been suggested to regulate calcium homeostasis (Aurora et al., 2012). These miRs can ultimately play an important role in regulating cardiac contractility, which is dependent on calcium signalling. Indeed, miR-mediated changes in calcium handling and signalling, by overexpression/knockdown of specific target miRs, could represent a new therapeutic promise (Harada et al., 2014).

Another study examined miR expression in patients undergoing remote IPC for the purpose of cardioprotection during surgery (Slagsvold et al., 2014a), randomising CABG patients to a remote IPC (n=30) or a control group (n=30). Results from analysing right atrial appendage samples indicated that miR-133a and miR-133b were increased in both groups after aortic cross-clamping, miR-1 was upregulated in controls, and miR-338-3p was increased in the IPC group. However, to the best of our knowledge, miR-338-3p has not, as yet, been assigned a cardiovascular-relevant effect and is mainly known to regulate epithelial to mesenchymal transition in cancer (Chen et al., 2016).

3.2. Therapeutic angiogenesis

The process of formation of new blood vessels is referred to as 'angiogenesis' and this is crucial in the progression of post-ischemic blood flow recovery and wound healing, facilitating revascularisation and vascular repair (Ferrara and Kerbel, 2005). In general, therapies can impede or promote angiogenesis. While anti-angiogenic therapies are being explored in scenarios such as tumour proliferation (Folkman, 1996), pro-angiogenic therapies can prove beneficial for restoring compromised cardiovascular function, e.g. after

an acute ischemic event. MiRs might play an important therapeutic role, as indeed they can control the expression of anti- or pro-angiogenic factors (Wang and Olson, 2009). To investigate the potential beneficial effect of promoting angiogenesis in the diseases/infarcted heart, several miRs have already been studied. For instance, miR-26a has been associated with increased vascular smooth muscle cell proliferation (Leeper et al., 2011), miR-132 has been associated with increased growth factor signalling (Anand et al., 2010), and miR-126 has been associated with increased Vascular Endothelial Growth Factor (VEGF) signalling in endothelial cells (EC) (Wang et al., 2008).

An important study in this context focused on miR-92a, which was found to be up-regulated in ischemic tissue in a mouse hind-limb ischemia model as well as after induction of acute MI (Bonauer et al., 2009). The study showed that inhibition of miR-92a benefited the angiogenic response, resulting in functional recovery of the damaged tissue and improved left ventricular systolic and diastolic function.

A recent study focusing on miR-24 (Meloni et al., 2013) employed a local adenovirus-mediated miR-24 decoy delivery to promote angiogenesis and restore blood perfusion in the myocardium, and beneficial effects were noted in terms of reducing infarct size, inducing fibroblast apoptosis, and an overall improvement in global cardiac function. Such observations on the beneficial role of miR-24 are in agreement with an earlier study (Fiedler et al., 2011) that also found that inhibition of miR-24 can increase vascularisation and ameliorate global cardiac function post-MI, thus suggesting its therapeutic potential.

The involvement of different miRs in post-ischemic angiogenesis has been discussed not only in the context of MI, but also in the context of peripheral artery disease leading to limb ischemia (Caporali and Emanuelli, 2012), further strengthening the case for their therapeutic potential.

Therapeutic angiogenesis could indeed benefit from a targeted approach involving miRs, and such a therapeutic approach could take two forms, i.e. either involving the increase of one or more specific angiogenic miRs, or by inhibiting anti-angiogenic miRs (Kane et al., 2014).

3.3. Post-operative complications

Following surgery on cardiopulmonary bypass (CPB), a series of acute complications can occur. One of the most threatening and frequent is acute kidney injury (AKI), which is initiated by renal ischemia and inflammation (Rosner and Okusa, 2006). AKI is associated with an increase in morbidity and mortality (Patel and Angelini, 2014). AKI also affects the paediatric population and it has been noted that repeated AKI can lead to chronic kidney disease particularly in high-risk cases such as transplant patients (MacDonald et al., 2016; Riley et al., 2015). Cardiac surgery-associated AKI is therefore considered a life threatening post-operative complication and at present no pharmacological intervention is available for successfully preventing it. In this light, further insight into AKI after cardiac surgery as well as the identification of possible therapeutic targets would be highly desirable.

Research has started to address the prognostic and treatment potential of miRs. . In particular, a study developed in urine and plasma samples of cardiac surgery patients (n=120, of which n=39 with progressive AKI, n=41 with non-progressive AKI and n=40 controls) has identified miR-21 as an interesting target for AKI prediction/diagnosis (Du et al., 2013). Up-regulated miR-21 levels both in urine and plasma in the AKI patients were associated with AKI progression. MiR-21 has a pro-survival role in apoptosis, and has roles in inflammatory and pro-fibrotic signalling pathways in AKI (Li et al., 2013). A recent animal study (Lorenzen et al., 2011), in which MI was induced in a murine model to simulate AKI onset post cardiac bypass surgery in humans, noted that deletion of miR-150 led to reduced inflammation and reduced interstitial cell apoptosis. This suggested that inhibiting miR-150 could have a therapeutic potential in patients.

Aortic surgery patients are also at risk of neurological injury by prolonged deep hypothermia circulatory arrest. Inhibition of miR-29c was suggested as protective for the neurological function in a rat study (Wang et al., 2015). Albeit preliminary, these observation hold promise for future exploration (Squiers et al., 2015) as neurological injury is a devastating complication after cardiovascular surgery and few pharmacological agents or interventions

are available to effectively protect the brain and spinal cord in the postoperative period (Torres and Ishida, 2015). In vivo studies have further suggested a neuroprotective role for antagomiRs against miR-145, miR-497, miR-181a, and miR-1 (Maegdefessel, 2014).

Another possible complication after cardiac surgery is pulmonary injury, whereby the deleterious effects of CPB combined with patient factors such as smoking or pneumonia compromise lung function (Clark, 2006). Pulmonary dysfunction is a frequent complication after cardiac surgery, often manifesting itself as pulmonary oedema, and in severe manifestations it can lead to acute respiratory distress syndrome, which has a mortality rate > 50% (Schlensak and Beyersdorf, 2005). A recent study (Yang et al., 2015a) performed miR microarray experiments to determine miR levels in blood samples from patients with acute lung injury induced by CPB, finding a significant correlation between the level of miR-320 and levels of TNF- α , respiration index and oxygenation index. In A549 cells, up-regulated miR-320 inhibited proliferation and increased apoptosis. Also, increased miR-320 levels resulted in lower expression of Silent mating type information regulation 2 homolog-1 (SIRT1) and the authors suggested miR-320 as a possible mediator for acute lung injury after CPB.

4. MicroRNA therapeutics

4.1. Concepts of microRNA therapeutics

Because of their relevance in disease, their known sequence and pharmacological properties, miRs are attractive candidates for therapeutic manipulation, including for different cardiac surgery scenarios. Based on lessons learned from antisense technologies, it has been relatively easy to develop and synthesize oligonucleotide chemistries to therapeutically regulate miRs. In contrast to a classical drug approach, miR-therapeutics are designed knowing that they will affect all genes that are under the control of the target miR. Since individual miRs often target numerous related mRNA genes that encode multiple components of complex intracellular networks, the regulation of a single miR can have a profound impact on cellular phenotypes. Pharmacological inhibition of individual miRs can be

achieved by modified antisense oligonucleotides, referred to as antimiRs, while miR replacement can be achieved by miR mimics to increase the level of a specific miR (van Rooij and Kaupinnen, 2014).

Restoring the function of a lost or down-regulated miR can be realized by either a miR mimic or by viral-mediated delivery of a miRNA. MiRNA mimics are synthetic RNA duplexes harbouring chemical modifications that improve their stability and cellular uptake while mimicking the endogenous functions of the miRNA of interest. Since these compounds need to be recognized by the miR processing system as being a miR, the allowed chemical modifications so far have been limited. Therefore high and repeated doses are currently required to establish sufficient miR delivery (Montgomery et al., 2014). Further optimization of the chemistry, local delivery or delivery vehicles will be required to translate these findings into the clinic. Data from the first Phase 1 study of the liposome-formulated miR-34 mimic-based drug (Bouchie, 2013) were announced in the end of 2015 and further in summer 2016, showing safety and dose-dependent effects on miR-34 target genes in white blood cells of primary liver cancer or metastatic cancer with liver involvement (www.mirnarx.com). Recently, the clinical trialling of a second mimic for miR-29b has shown decreased expression of collagen and other proteins that are involved in fibrous scar formation and that represent direct targets of miR-29 (van Rooij et al., 2008). After testing in healthy individuals, the study will advance into patients with cutaneous scleroderma to investigate the safety and tolerability of miR-29b mimic when injected into active fibrotic lesions (www.miRagenrx.com). AntimiRs are modified antisense oligonucleotides that can inhibit the levels of disease-driving or aberrantly expressed miRs. Because miRs typically act as inhibitors of gene expression, antimiRs will result in a de-repression of the mRNAs that are normally targeted by the miR. The use of anti-miRs involves using artificial miR target sites to inhibit endogenous miR regulation (Brown and Naldini, 2009; van Rooij and Olson, 2012). Prior to actually performing a clinical trial on an anti-miR, insight into the anti-miR drug is gathered through the necessary steps of optimisation of suitable drug candidates, performing

pharmacokinetics, pharmacodynamics, and absorption, distribution, metabolism and excretion studies, as described in detail elsewhere (van Rooij et al., 2012).

An efficacious antimiR requires for it to be stable *in vivo*, with a high specificity and binding affinity to the miR of interest. This can be achieved with chemical modifications. The most commonly used modifications are 2' sugar modifications such as 2'-O-methyl (2'-OMe), 2'-O-Methoxyethyl (2'-Moe), 2'-fluoro (2'-F), or locked nucleic acid (LNA). Additionally the balance between phosphodiester (PO) and phosphorothioate (PS) linkages between the nucleotides also influences stability by nuclease resistance and facilitation of cellular uptake. PS backbone linkages, whereby sulfur replaces one of the non-bridging oxygen atoms in the phosphate group, are more resistant to nucleases than PO, and thereby providing more stability to the oligonucleotide. Moreover, increasing the abundance PS backbone modifications promotes plasma protein binding, thereby reducing clearance by glomerular filtration and urinary excretion, which facilitates tissue delivery of antimiRs *in vivo*.

While the first pre-clinical studies used cholesterol-conjugated oligonucleotides targeting the full miR, currently the preferred chemistry are the shorter LNA-modified LNA/DNA mixmers. Many *in vivo* pharmacokinetic studies have shown that these compounds can be delivered subcutaneously and will distribute to all organs, including the cardiovascular system, with a higher exposure to the kidney and liver (Hullinger et al., 2012). Also the first antimiR drugs have entered the clinical arena. Santaris pharma showed both safety and efficacy of their antimiR against miR-122, miravirsen, in humans (Janssen et al., 2013; van der Ree et al., 2014). These data indicated that miravirsen given as a four-week monotherapy to HCV patients provides long-lasting suppression of viremia and provides a high barrier to viral infection. Additional clinical trials using GalNAc-conjugated antimiR-21 and antimiR-103/107 oligonucleotides were recently started with the aim of treating Alport syndrome, a genetic kidney disease, or patients with metabolic diseases such as type 2 diabetes and NASH (www.Regulusrx.com). Although there are currently no clinical trials for cardiovascular disease on-going, the positive advancements in other areas of disease support the great enthusiasm for exploring miR therapeutics as a new class of drugs for cardiovascular indications.

4.2. Delivering microRNA therapeutics

The main delivery methods for miR therapeutics discussed in the literature involve lipid-based vehicles, viral systems, and cationic polymers (Di Pasquale et al., 2012; Giacca and Zacchigna, 2012; Nouraei and Mowla, 2015). Adeno-associated viruses can be alternative and possibly more efficient media, particularly for targeted miR therapeutics (Santulli et al., 2014). Two main strategies can be used to inhibit miRs in the heart, namely using either 2'-O-methyl group-modified oligonucleotides or LNA-modified oligonucleotides, with the anti-miRs injected either intravenously or subcutaneously (Bernardo et al., 2012). Beneficial intravenous or subcutaneous injection of antagomirs has been discussed in the literature (Bonauer et al., 2009; Montgomery et al., 2011). Nevertheless, using anti-miR oligonucleotides as a potential therapeutic tool can be associated with the risk of affecting RNA species beyond the intended target (Stenvang et al., 2012) and using LNA-containing anti-miRs can block the expression of miRNAs from the same family depending on the specificity of the seed region (Li and Rana, 2014).

Local delivery is desirable for miR therapeutics, in order to target the myocardium, a specific part of the vasculature, or heart valves. Moreover, protecting the organs damaged by surgery is also desirable. Exciting concepts include the development of devices specific for local delivery, as possibly novel biodegradable scaffolds (Hastings et al., 2015). Light-induced anti-miR activation has been discussed as another method, and this has been presented as a viable option in the context of suppressing the anti-angiogenic miR-92a in order to improve angiogenesis (Schäfer et al., 2013). This study in particular suggested that the use of light-inducible anti-miRs could facilitate local activation, overcoming a limitation of systemic inhibition of miRs, i.e. possible side effects and oncogenic effects. This method of delivery looks compatible with open-heart surgery; indeed, cardiac surgery might be the ideal setting to test its validity at clinical level.

Another method for delivery could be represented by a new generation of minimally-invasive (i.e. delivered via catheter) biodegradable scaffolds. Studies in the area of material

properties aimed at identifying the appropriate biochemical composition and structural properties could then be coupled with innovative designs in order to create miR-releasing devices that could be used also for treating aneurysms or calcifications. With regards to controlled release of therapeutic agents (including miRs) with stent-based methods, i.e. gene eluting stents, it has been suggested that delivery could involve the complexing of a siRNA/miR molecule with cationic polymers, which is then loaded onto the stent surface (Laçin et al., 2015; Santiago and Khachigian, 2001). The use of a pH-sensitive Upy hydrogel has also been suggested for cardiac delivery of miR therapeutics (Hastings et al., 2015).

Another novel and appealing method for potentially delivering miRs for therapeutic purposes involves the use of bioengineered exosomes of endogenous nature or “artificial” exosomes (namely nanoparticles mimicking exosome composition). Exosomes are very small extracellular vesicles which have the capacity to transport a cargo, including mRNAs, miRs, actins, proteins, enzymes, molecular chaperones and signalling molecules (Emanuelli et al., 2015). So-called ‘exosome-encapsulated’ miRs play a role in the cardiovascular system (Shantikumar et al., 2014) and could also serve as therapeutic delivery agents. Indeed, the appealing idea of a “possible therapeutic Trojan Horse” to deliver biological therapeutics, including short interfering RNAs and recombinant proteins” has been put forward (Emanuelli et al., 2015).

Advantages of miR therapeutics over molecular therapeutics include their small size, preserved sequences and stability in fluids (Nouraei and Mowla, 2015). The use of exosomes or nanovesicles for delivery therapeutics has the advantages of facilitating transfer across biological barriers and avoid degradation that might occur if such therapeutics were delivered in the circulation (Suntres et al., 2013). A schematic summary possible beneficial effect of miR for cardiovascular therapeutics and associated delivery methods is presented in Figure 4.

5. MicroRNAs as clinical biomarkers

Albeit beyond the main focus of this Review, the potential role of miRs as biomarkers in cardiovascular disease and cardiac surgery patients deserves attention, and it is worth mentioning it briefly as it applies to several of the scenarios that have been discussed so far. The role of miRs as diagnostic biomarkers has been extensively explored in HF and MI patients (Gao et al., 2015; Gidlöf et al., 2013; Goren et al., 2012; Lai et al., 2015; Vogel et al., 2013) and a recent meta-analysis (Cheng et al., 2014) confirmed that “the correlation between miRs and other diagnostic biomarkers of myocardial infarction [is] obvious”, particularly so for miR-499 and miR-133a. With regards to open-heart surgery and CABG surgery, recent work has demonstrated a significant increase in plasma concentrations of exosomes and their cargo of cardiac miRs in CABG patients, suggesting trafficking of exosomes from the heart into the circulation post CABG (Emanuelli et al., 2016). Measurements during open-heart surgery revealed variations in miR levels, including miR-1, miR-208a and miR-499 during surgery, these levels in turn being associated with changes in creatine kinase-muscle band and cardiac troponin (Emanuelli et al., 2016; Yang et al., 2015b). These results again suggest the potential of miRs as biomarkers, particularly for ischemia and reperfusion injury during open-heart surgery. Observations in this context are, however, not entirely in agreement. A slightly larger prospective study in surgical CAD patients observed decreased miR-133 expression in right atrial myocardium samples is associated with various characteristics of HF, but no significant changes in miR-1, thus generally implying a role for miR-133 but not miR-1 in the ischemic heart remodelling process (Danowski et al., 2013). A third study (Slagsvold et al., 2014b) used LV biopsies from 60 CABG patients randomised in a remote IPC group and a control group, but found no difference in miR expression between the two groups. Indeed, it has been pointed out that larger studies on well-matched populations could shed further light on the role of miRs as possible biomarkers specifically for I/R injury in cardiac surgery patients (Caputo et al., 2015b). Moreover, circulating miR-499 has been proposed as possible biomarker for perioperative MI in CABG patients operated “on pump” (i.e., using CPB) (Yao et al., 2014). Higher miR-21 levels in atrial tissue samples have been linked to the presence of atrial

fibrosis and thus is a possible biomarker for atrial fibrillation in cardiac surgery patients (Nishi et al., 2013). A smaller study also reported that variations in circulating miRs could convey information about early myocardial injury post heart transplant, suggesting miR-133a, miR-208a and in particular miR-133b as potential markers (Wang et al., 2013).

In the context of aortic stenosis and AVR, a recent study concluded that “circulating miR profiling requires further refinement before translation into clinical use as a biomarker in aortic stenosis” (Coffey et al., 2015). On the other hand, a blood test yielding information on circulating miRs could possibly provide useful biomarkers for differentiating subtypes of thoracic aortic aneurysm (Ikonomidis et al., 2013). In this case, both thoracic aortic aneurysm and aortic dissection are associated with high mortality and high cost; from a biomarker perspective, the benefit of identification of high-risk patients based on their miR expression can be clinically valuable for risk stratification, hence following some patients more frequently, or even performing prophylactic surgery for those at higher risk (Zhang et al., 2013b). A previously mentioned study (García et al., 2013) identified miR-133a as a predictor of LV hypertrophy and its reversibility following AVR, highlighting the possible role of this miR for providing additional information on surgical timing in asymptomatic aortic stenosis. In aortic aneurysm cases, miR-195 has been indicated as a potential biomarker, as its plasma levels appeared to be reduced in patients with aneurysmal aortas, likely related to its effect on the extracellular matrix (Zampetaki et al., 2014).

Another study observed up-regulation of miR-210 in AKI patients, and a significant reduction in miR-320 and miR-16 (Lorenzen et al., 2011). Also, miR-210 was found to be an independent and significant predictor of 28-day survival in AKI patients. The study looked at circulating miRs and did not establish a role of such miRs in the progression of AKI; indeed, as discussed elsewhere (Molitoris and Molitoris, 2011), it is difficult at this stage to argue the specificity of such findings considering the systemic multi-organ involvement that could affect observations in this population. Nevertheless, circulating biomarkers could have an important diagnostic role, as the early diagnosis of AKI remains challenging (Bellinger et al., 2014).

6. Translational outlook and conclusions

The field of miR research has touched upon several areas that are relevant to cardiac surgeons, particularly for CABG and aortic aneurysms, but it has also explored the complex area of congenital defects and their surgical repair. It is now considered that miRs play a central role in cardiovascular disease (Olson, 2014) and, although the majority of the studies present very insightful yet predominantly descriptive data, the possibility of targeting miRs for therapeutic purposes could be revolutionary in the surgical field (Epstein, 2010). The very nature of cardiac surgery affords a fortuitous opportunity for the delivery of miR therapeutics.

From a translational perspective, the development of suitable cardiac surgery animal models would represent an important step toward testing novel miR therapeutics, particularly involving large animal models. The latter can provide a more suitable setting to simulate surgical practices and/or replicate scenarios, e.g. large animal models in AKI research (Ghorbel et al., 2014), instead of murine models that are unlikely to mimic sufficiently the pathology of the surgical scenarios of interest (Emanuelli and Angelini, 2015).

On the other hand, the role of miRs as biomarkers is equally important and can be tremendously insightful. In this context, the role of circulating miRs could be particularly valuable as diagnostic or even prognostic biomarkers (Fichtlscherer et al., 2011). Another exciting goal for future research is to improve our knowledge of pathophysiological mechanisms underlying cardiovascular conditions, possibly by merging miR expression with functional data from medical imaging, similar to a recent, elegant study that merged imaging information with local tissue sample analysis (Guzzardi et al., 2015).

The development of miR-based therapeutics for cardiac surgery still requires more analyses to be carried out directly on samples obtained from cardiac surgery patients across the different populations described in this review, along with work on suitable large animal

models, and ultimately, investigations into efficacy and safety. This needs to be carried out in parallel with pharmacokinetic and pharmacodynamic studies of potential novel miR-based compounds.

7. Conflict of interest statement

The authors declare that there are no conflicts of interest. EvR is co-founder and scientific advisor of miRagen Therapeutics, Inc.

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Table 1: Summary of relevant miRNAs in cardiac surgery patients

Surgical context	Relevant process	Involved miRNA(s)	Reference
The ischemic heart	Atherosclerotic plaque formation	miR-21, -27, -92a, -143/145	Caroli 2013
	Ischemia and cardiac repair	miR-15	Small 2010; Porrello 2011
	Improved angiogenesis and inhibition of cardiac apoptosis	miR-210	Hu 2010
	Stimulation of cardiomyocyte proliferation	miR-199a, -590	Eulalio 2012
LVAD support	Ventricular remodelling	miR-1, -133a, -133b	Schipper 2008
Aortic valve replacement	Improved LV remodelling (regulating apoptosis, fibrosis, and potassium channels remodelling)	miR-133	Garcia 2013; Care' 2007
	Aortic valve calcification	miR-141	Yanagawa 2012
		miR-30b	Zhang 2014
Aneurysmal aorta	Aneurysm development	miR-1, -21, -29a, and -133a	Ikonomidis 2013; Jones 2011
	Aortic dissection	miR-29, -30	Liao 2011
Congenital heart disease	Embryonic development	miR-1	Bruneau 2008
Acute kidney injury	Pro-survival role in apoptosis, and inflammatory and pro-fibrotic signalling pathways in AKI	miR-21	Li 2013
	Inflammation and apoptosis	miR-150	Lorenzen 2011

Figure legends

Figure 1: Schematic summary of different microRNAs (miRs) playing a role in cardiac surgery patients. CABG = coronary artery bypass graft; AVR = aortic valve replacement.

Figure 2: Schematic summary of different microRNAs (miRs) playing a role in congenital heart disease. PAH = pulmonary arterial hypertension secondary to congenital heart disease; BAV = bicuspid aortic valve; ToF = tetralogy of Fallot; TGA = transposition of the great arteries; HLHS = hypoplastic left heart syndrome. Note: the role of miR-423-5p in TGA was investigated but no conclusive evidence was found. It is, nevertheless, included in the figure for completeness.

Figure 3: Schematic summary of different microRNAs (miRs) playing a role in mechanisms relevant in cardiac surgery patients, including post-operative complications, angiogenesis and myocardial protection.

Figure 4: Schematic summary of possible beneficial effect of microRNAs for cardiovascular therapeutics, including delivery methods.

Figure 1

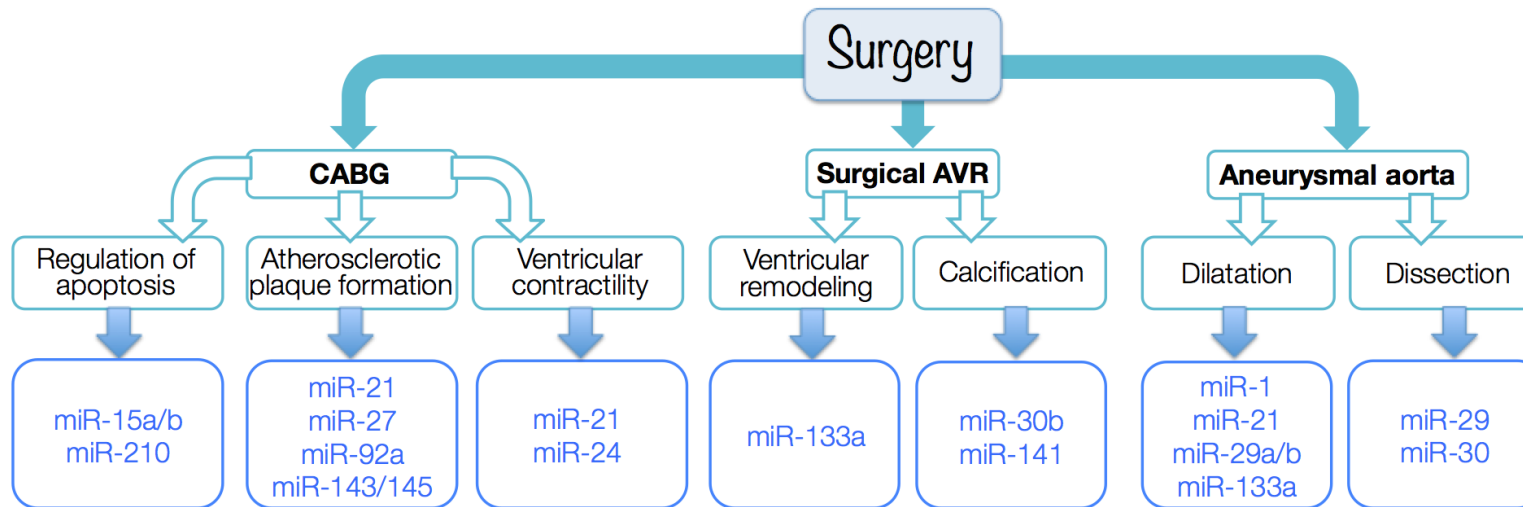


Figure 2

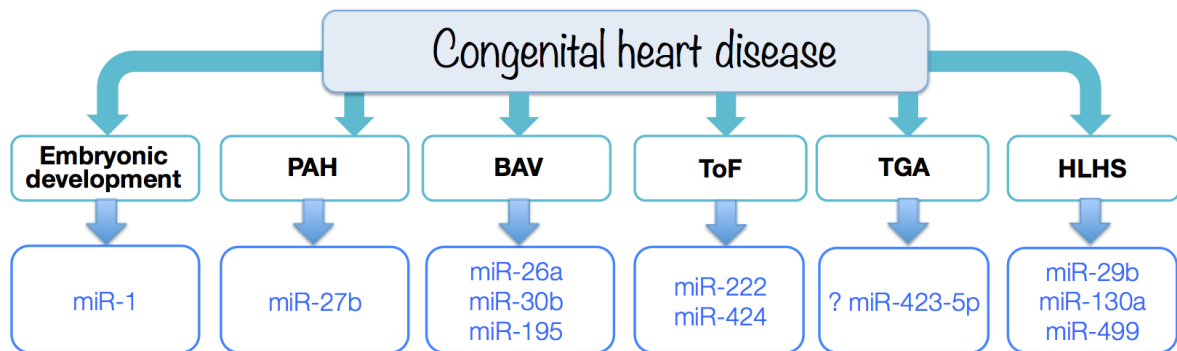


Figure 3

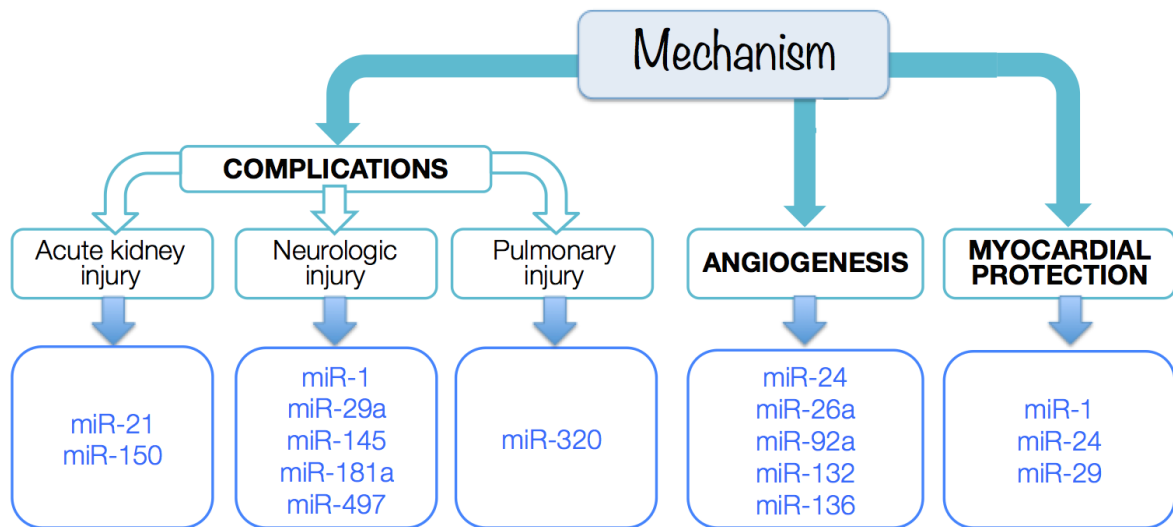


Figure 4

